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Enzymatic hydrolysis of polyester based coatings

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ABSTRACT

The potential of two hydrolytic enzymes, namely a lipase from *Thermomyces lanuginosus* (TlL) and a cutinase from *Humicola insolens* (HiC) for hydrolysis of the phthalic acid backbone based polyester coatings was assessed. Two phthalic acid/trimethylolpropane based model substrates resembling the structure of the polyester backbone of coatings were synthesized. Out of both enzymes, only the cutinase was able to hydrolyze both model substrates while the larger substrate was hydrolyzed at a lower rate. The cutinase was also able to hydrolyze a coating (alkyd resin) both in suspension and as dried film. LC–MS analysis of the hydrolysis products released from the coating revealed the presence of oleic acid, its monoglyceride, phthalic acid and 2-((3-((2-((2,3-dihydroxypropoxy)carbonyl)benzoyl)oxy)-2-hydroxypropoxy)carbonyl)benzoic acid. These results indicate that the enzyme was able to hydrolyze the polyester backbone as well as to release fatty acid side chains. Consequently, enzymatic hydrolysis has a potential for the removal of coatings, their recycling or their functionalization.

1. Introduction

Various types of polyesters are important components of coating materials. These include among others oxidatively drying alkyd resins and waterborne oil-free polyesters. Alkyd resins consist of a polyester backbone of polyalcohols, dicarboxylic acids or anhydrides and unsaturated fatty acids or triglycerides that are esterified to the backbone [1]. Alkyd resins were developed in the 1920s and are today used in different fields like road marking, house and decorative paints with an annual worldwide production of several million tons [2]. Additionally, they act as protection coatings for wood materials against several environmental impacts like radiation [3,4]. Waterborne oil-free polyesters are produced via polycondensation of polycarboxylic acids (e.g. phthalic acid) and polyalcohols with a concomitant elimination of water [3]. Waterborne polyesters are extensively used for architectural paints [5].

In case of both phthalic acid based waterborne oil-free polyesters and alkyd resins, removal of coatings required in renovation processes is quite difficult while recycling of individual components is currently almost impossible [6]. Similarly, recycling of coating production wastes or cleaning of machinery is quite problematic. Enzymes could catalyze the hydrolysis of coatings into their individual components under environmentally friendly conditions. Moreover, partial surface targeted hydrolysis could introduce new reactive groups [7] allowing easier coating/grafting of further functional layers.

Ever since the most recalcitrant polyester – polyethylene terephthalate (PET) – was found to be susceptible to enzymatic hydrolysis [8], various different types of enzymes ranging from lipases [9,10], esterases [11] to cutinases [12–16] have been described to hydrolyze this complex synthetic substrate. Among all these enzymes, undoubtedly most studies used cutinases for polyester hydrolysis [12]. These enzymes hydrolyze cutin, a complex polyester mixture constituent of the cuticle, which covers aerial tissues of vascular plants [17]. This natural polyester composite consists mainly of C_{16}–C_{18} esterified oxygenated fatty acids [18], and can be hydrolyzed by cutinases, which are secreted during the first step of plant infection.

The development of model substrates [19] mimicking the structure of the targeted polymer allowed a faster screening of potential candidates and has contributed to the identification of enzymes with potential to hydrolyze synthetic polymers like PET [20–22] or polyamides [23,24]. In this paper we have designed new model substrates resembling the structure of phthalic-acid based polyester coatings and we show for the first time that phthalic acid based alkyd resins can be hydrolyzed/functionalized with enzymes.

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2. Experimental section

2.1. Materials and methods

2.1.1. Chemicals and enzymes

Acetonitrile (LC–MS grade), formic acid and THF (LC–MS grade) were purchased from Sigma Aldrich. *Humicola insolens* Cutinase (HiC) and the *Thermomyces lanuginosus* Lipase (TlL) were from Novozymes (Denmark) and INOTEX (Czech Republic. Both enzymes were liquid preparations that were used without any further purification. The alkyd resin was kindly synthesized from Cytec Surface Specialties, (Austria).

2.1.2. ACID NUMBER measurements

The acid number was measured according to DIN EN ISO 3682 and is defined as the quotient of the mass of potassium hydroxide (KOH) that is necessary to neutralize the sample to be analyzed and the mass of the sample.

2.1.3. Synthesis of PTa and PTb model substrates

About 1.0 mole of trimethylolpropane were melted and heated to 120 °C under stirring. 0.1 g of triethylamine were added as a catalyst, followed by a stepwise addition of 1.0 mole of phthalic anhydride within 30 min. Afterwards, the temperature was raised up to 135 °C and kept constant until an acid number of 198 mg KOH/g was reached. In the case of PTb, the temperature was kept constant until the acid number decreased to 120 mg KOH/g followed by a further increased to 160 °C until an acid number of 70 mg KOH/g was reached. Finally dipropylene glycol dimethyl ether (DPGDME) was added to both preparations as a solvent until a solid content of 80% was reached.

2.1.4. Gel permeation chromatography

The samples were dissolved in 5 ml THF and further diluted with THF containing 0.02% sulfur. Subsequently, they were filtrated using a Millipore glass fiber prefilter and a Millipore FluoroporeTM membrane filter (0.45 μm). GPC analysis was performed using an Agilent 1100 Isocratic Pump G 1310A, an Agilent 1100 Autosampler G 1313A and an Agilent RID 1200 G 1362A (Agilent Technologies, USA) on 2 sequenced Polymer Labs PL Mixed C columns (5 μm, 7.8 mm × 300 mm). THF (containing 5 g L-1trifluoroacetic anhydride) was used as mobile phase with a flow rate of 1.0 ml min^{-1}. The calibration was performed with polystyrene standards. Data were collected and analyzed using Polymer Standards Service WINGPC Unity software.

2.1.5. Titration

Hydrolysis of model substrates and alkyd resin was assayed by using a pH stat titration instrument (Mettler Toledo, Germany). The titration studies were performed in a final volume of 25 ml, which was pre titrated to pH = 7 with NaOH (0.1 M), before starting the hydrolysis. In the case of the model resins PTa & PTb, they were initially dissolved in DPGDME 100 mg in 25 ml. This solution was further diluted 1–4 with water and used for the assay. In the case of the alkyd resin, the assayed solution consisted of a dilution 1:10 of alkyd resin. The reactions were started by adding the respective enzyme. Final concentrations were 100 μg/ml or 200 μg/ml. Initial hydrolysis rates were calculated and expressed in μmol of NaOH per gram of substrate and hour. All the experiments were performed in duplicates. Control samples without enzyme for each substrate were carried out as blank and the background hydrolysis values were deducted from the enzymatic hydrolysis values.

2.1.6. Hydrolysis reactions for analysis of reaction products

Hydrolysis reactions were performed by incubating the polyester substrates with HiC and TlL at 50 °C for 6 h at 600 rpm. The substrates PTa and PTb were first dissolved in DPGDME as for the titration studies, before adding the enzyme (400 μg/ml) to the reaction mixture (containing 0.9 mg/ml polyester model substrate). The alkyd resin was not diluted previous to the hydrolysis reaction.

2.1.7. HPLC–MS

To the incubation mixtures (see hydrolysis) an equal volume of ice-cold methanol was added in order to precipitate the enzyme. The mixture was then centrifuged for 10 min at 14,000 g to remove precipitated enzyme. HPLC analysis was performed using a system from Dionex equipped with a P580 pump, an ASI-100 autosampler and a PDA-100 photodiode array detector while monitoring elution at 254 nm. Separation of coupling products was achieved by reversed phase HPLC RP-C18 column (XTerra, Waters) using 60% H_2O, 20% acetonitrile and 20% formic acid (0.1%) as solvent at a flow rate of 0.4 ml min^{-1}, an injection volume of 10 μl and an oven temperature of 25 °C. The MS spectra were acquired with an Agilent Ion Trap SL (Palo Alto, CA, USA) equipped with an electrospray ionization coupled to the Dionex HPLC-UVD-system as described above and initial separation of coupling products was carried out using the same protocol. The coupling products were measured in positive ion mode and the electrospray voltage was set to +3500 V. Dry gas flow was set to 10 l min^{-1} with a temperature of 350 °C, nebulizer set to 50 psi. Maximum accumulation time was fixed to 300 ms and the loading of the trap was controlled by the instrument with an ICC of 30,000.

3. Results and discussion

In this study, the potential of enzymes to hydrolyze phthalic acid based polyester coatings was assessed. For this purpose, enzymes hydrolyzing natural polyesters like cutin (cutinases) and enzymes hydrolyzing natural triglycerides (lipases) were studied [25]. Based on previous studies on PET hydrolysis, a cutinase from *Humicola insolens* [21] together with a lipase from *Thermomyces lanuginosus* [26] were selected to investigate hydrolysis of phthalic acid based polyester coatings.

For this purpose, in a first step, two phthalic acid based polyester model substrates were synthesized. Trimethylolpropane was used as alcohol component, which is commonly used coatings but does not carry bulky substituents like acylglycerol moieties in alkyd resins which may reduce accessibility of the polyester backbone to enzymes. Defined model substrates for polymers are helpful tools for faster screening of new enzymatic activities [14,19] as well for mechanistic investigations regarding their mode of action [20,22,27].

Expectedly GPC analysis of the synthesized model substrates indicated a higher degree of polymerization after longer reaction time (Fig. 1). The average molecular weight determined for PTa was 5.4×10^2 g/mol compared to 1.5×10^3 g/mol measured in the case of PTb. In the GPC chromatogram (Fig. 2), it clearly can be observed that most of the compounds detected for PTa have a molecular weight lower than 1000 g/mol. Among the three main peaks detected the trend is that lower molecular weight substances are predominant, decreasing the abundance with increasing the size. For PTb the three predominant peaks detected in the case of PTa were also observed. However the peak size for all of them was significantly lower, being none of them the predominant one. In addition a big amount of not clearly defined substances, with molecular weight between 1000 and 5000 g/mol was measured. The latter broad peak confirmed that a higher degree of